REDUCTIVE BIOLOGICAL TREATMENT OF TEXTILE EFFLUENTS

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PORTUGAL

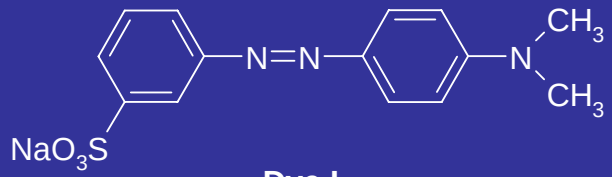
The beginning

- Collection of contaminated soil from a treatment plant that receives large amounts of coloured water
- Growth in rich medium to select microorganisms
- Isolation of yeasts in selective medium
- Growth of yeasts in YEPD-agar medium with a model azo dye to select strains with decolourising capability
- Identification of the yeasts with better decolourising activity: *Candida zeylanoides* (UM2) and *Issatchenkia occidentalis* (UM41)

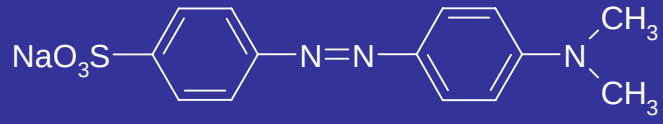
Study the decolourisation process by YEASTS

The decolourisation process by yeasts

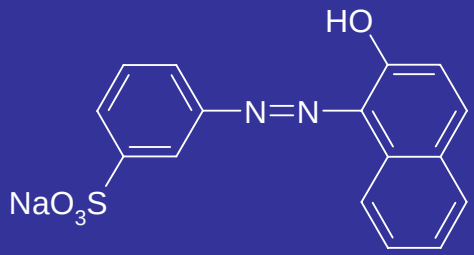
Selection of model azo dyes



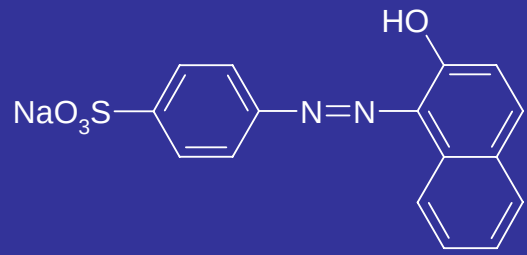
Dye I



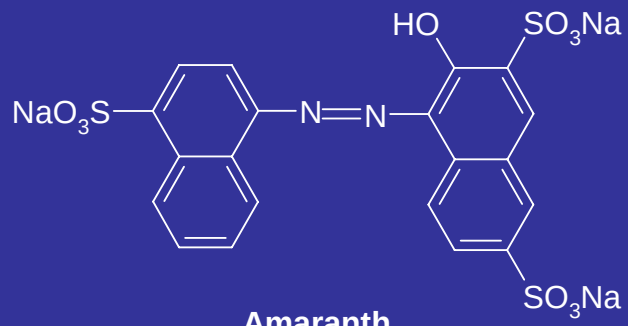
Dye II (Methyl Orange)



Dye III



Dye IV (Orange II)

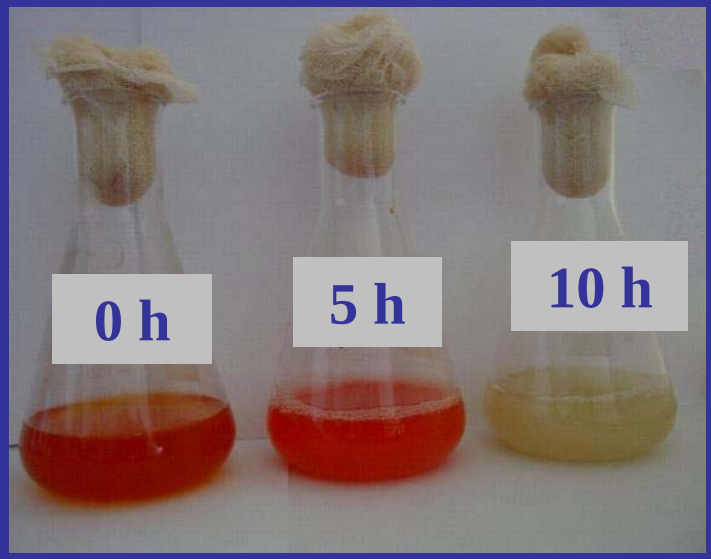


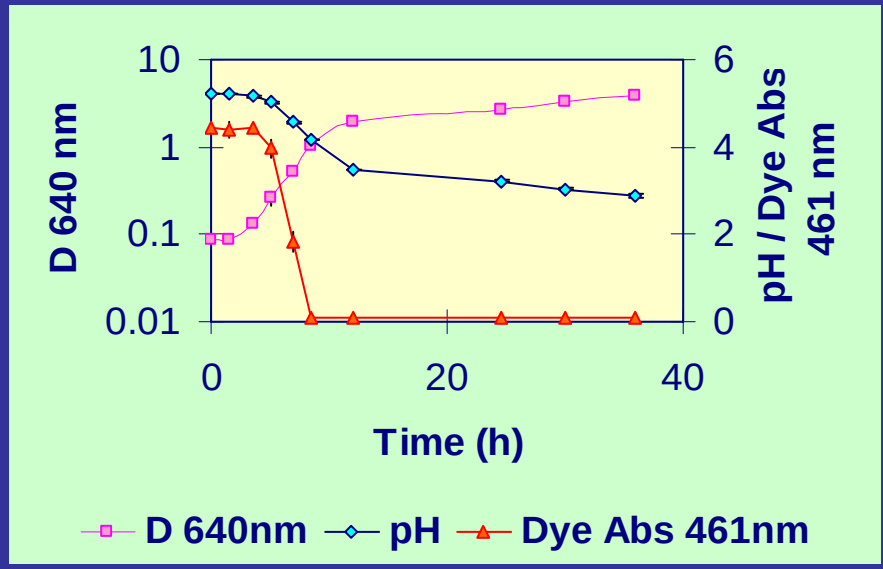
Amaranth

Ramalho et al. 2002 **Improved conditions for the aerobic reductive decolourisation of azo dyes by *Candida zeylanoides*** Enz Microb Technol 31: 848-854

The decolourisation process by yeasts

Decolourisation in batch:





Conditions:

- 0.2 mM dye
- NDM with 2% glucose
- 120 rpm
- 26ºC

Ramalho et al. 2002 **Improved conditions for the aerobic reductive decolourisation of azo dyes by *Candida zeylanoides*** Enz Microb Technol 31: 848-854

The decolourisation process by yeasts

Decolourisation in batch - results:

- The presence of dyes in the growth medium does not affect the specific growth rates;
- Yeasts are able to reduce azo dyes to colourless amines;
- This activity is constitutive;
- Depends on dye structure.

Ramalho et al. 2002 **Improved conditions for the aerobic reductive decolourisation of azo dyes by *Candida zeylanoides*** Enz Microb Technol 31: 848-854

The decolourisation process by yeasts

Decolourisation in batch - results:

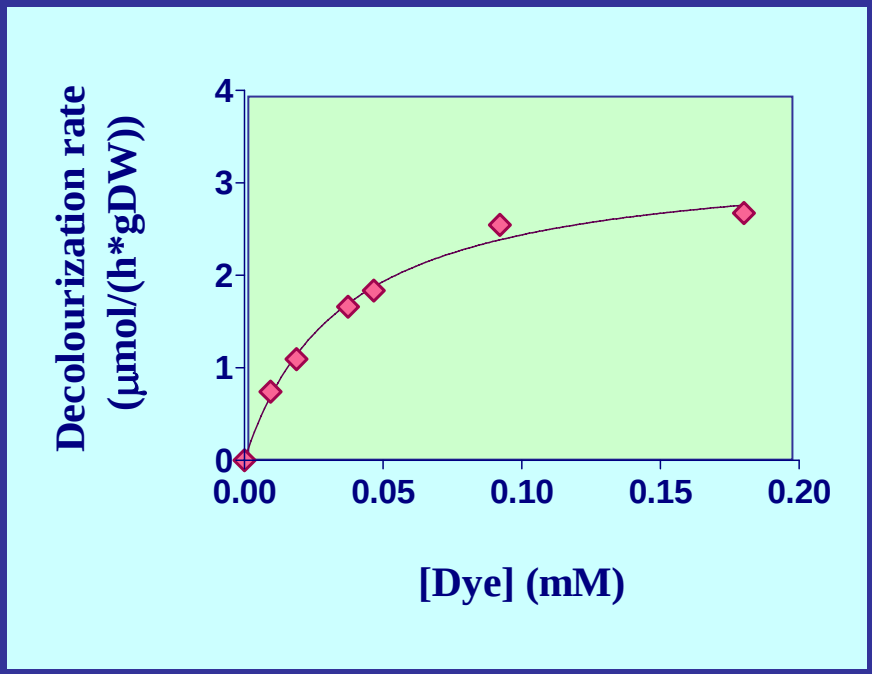
- Dye degradation is connected to growth phase;
- The glucose is fermented and the resulting ethanol is respired;
- O_2 in the incubation conditions is limited;
- Under anoxic or aeration conditions there is no decolourisation;
- Neither dyes nor resulting amines are toxic to yeasts;
- Yeasts can use the produced amines as carbon and nitrogen sources.

Ramalho et al. 2004 **Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain** Appl Environ Microbiol 70: 2279-2288

The decolourisation process by yeasts

Activity assays - results:

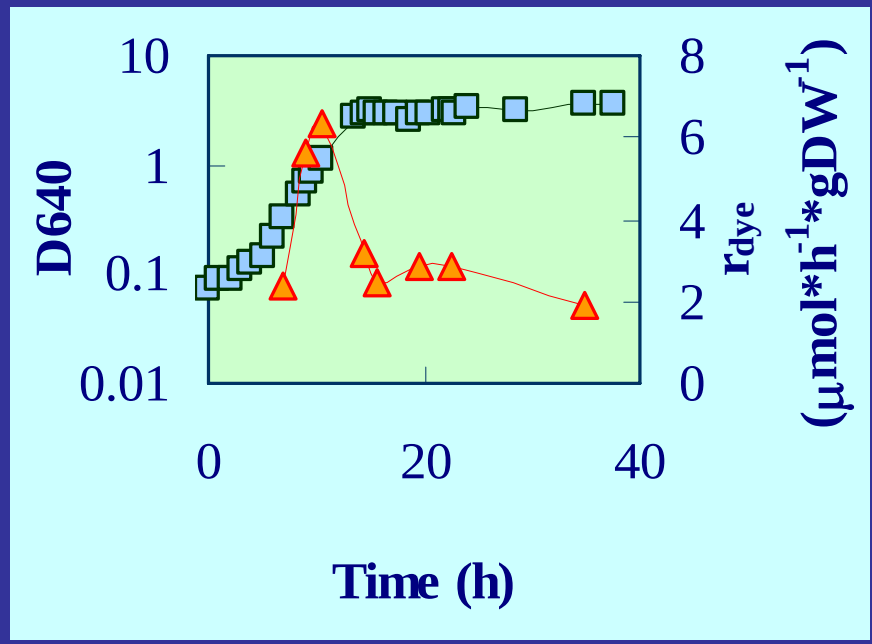
Effect of dye concentration



$r_{dye\ max}$ = 3.2 μmol*h^{-1}*g^{-1}

k_M = 0.034 mM

Effect of growth phase



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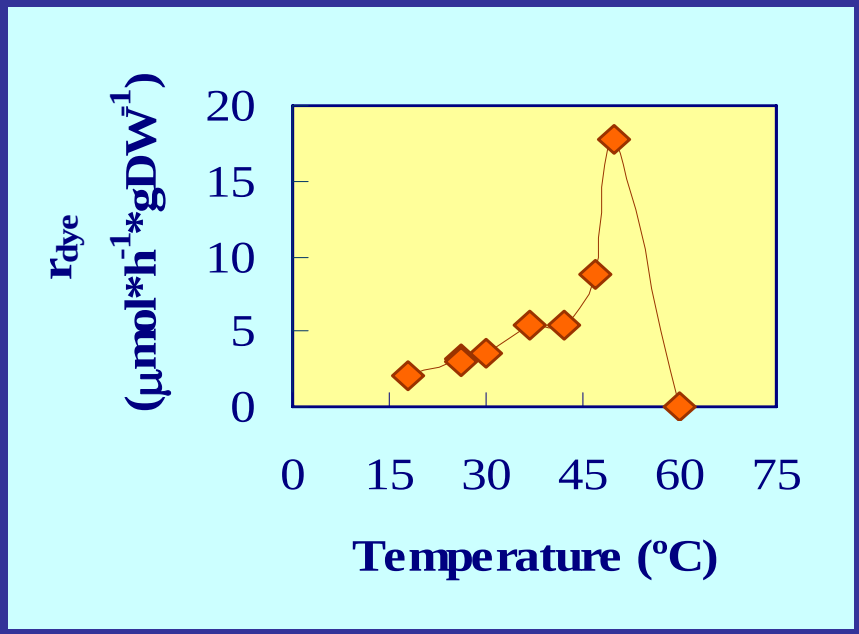
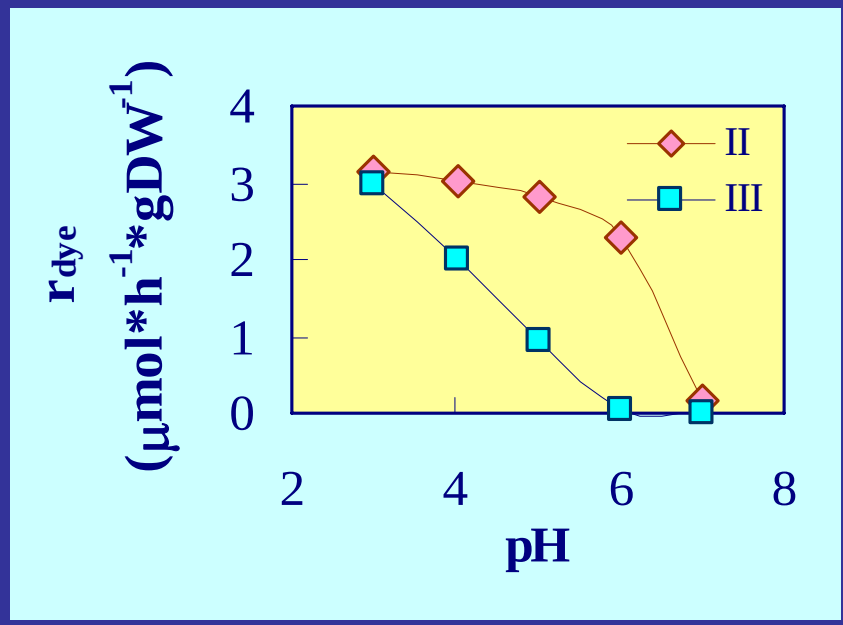
The decolourisation process by yeasts

Activity assays - results:

Effect of temperature



Effect of pH



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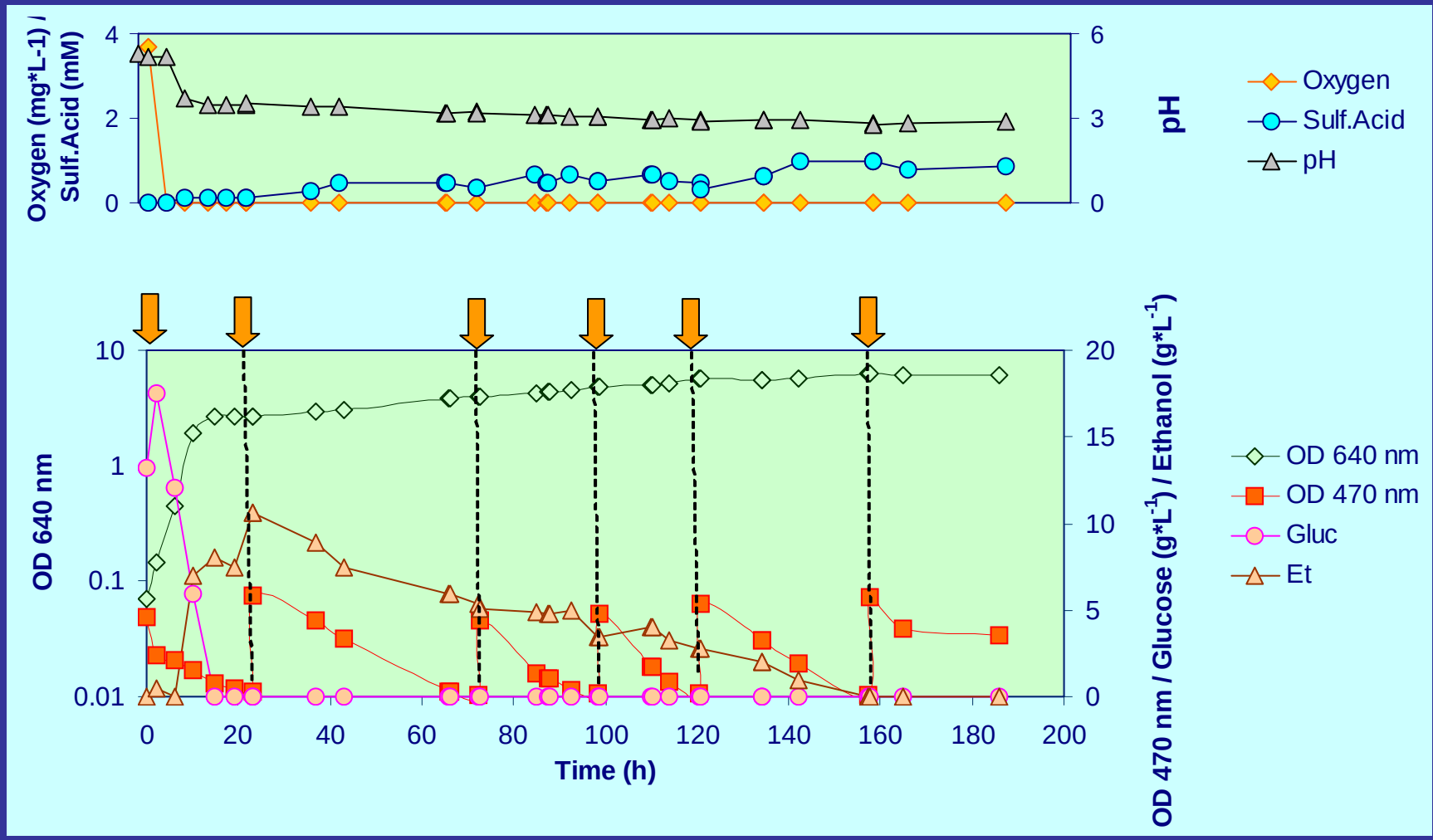
The decolourisation process by yeasts



Maximum capacity of decolourisation: 0.5 mM dye/ g glucose

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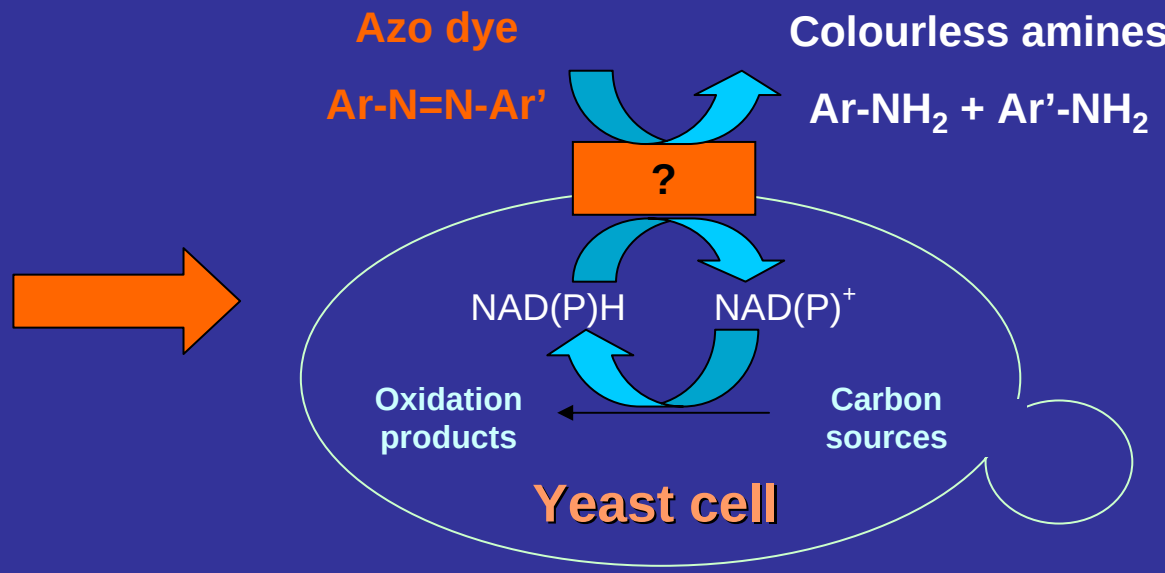
The decolourisation process by yeasts

Results:

- There is a need of an external carbon source;
- The activity is only detected in intact cells;
- Can use alternative carbon sources like acetate and ethanol.

Still unanswered question



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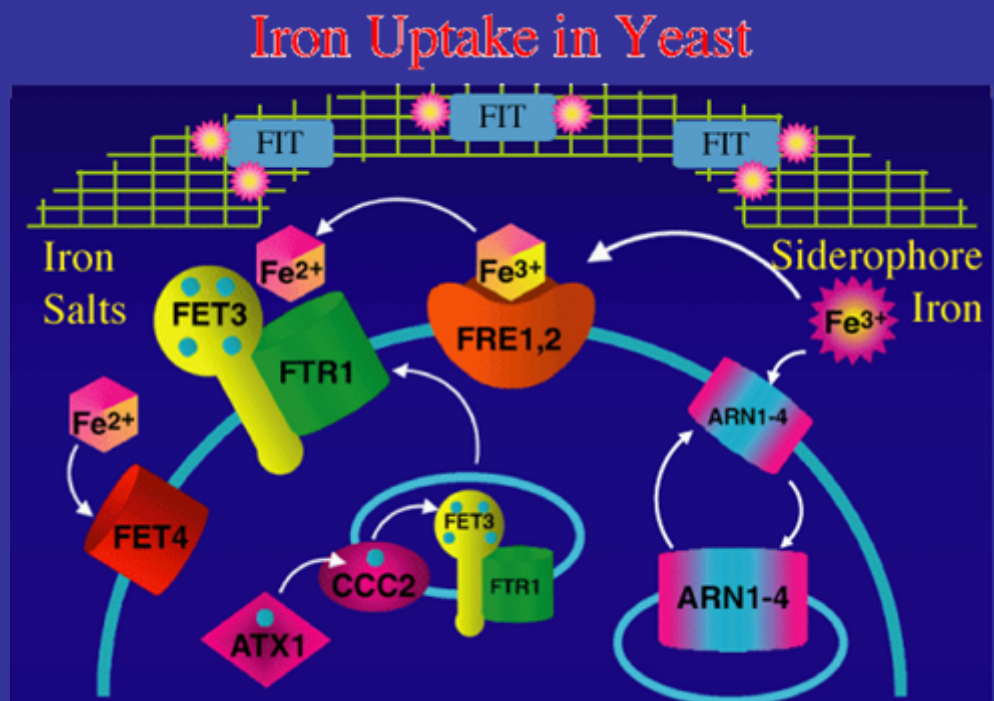
The enzyme system

From literature -> yeasts have a plasma membrane redox system to reduce iron(III) to iron (II) (solubilization of iron) – **THE FERRIC REDUCTASE SYSTEM**

Could this system be responsible for the azo reductase activity in yeast cells?

Sellection of laboratorial *Saccharomyces cerevisiae* strain with the ability to degrade azo dyes

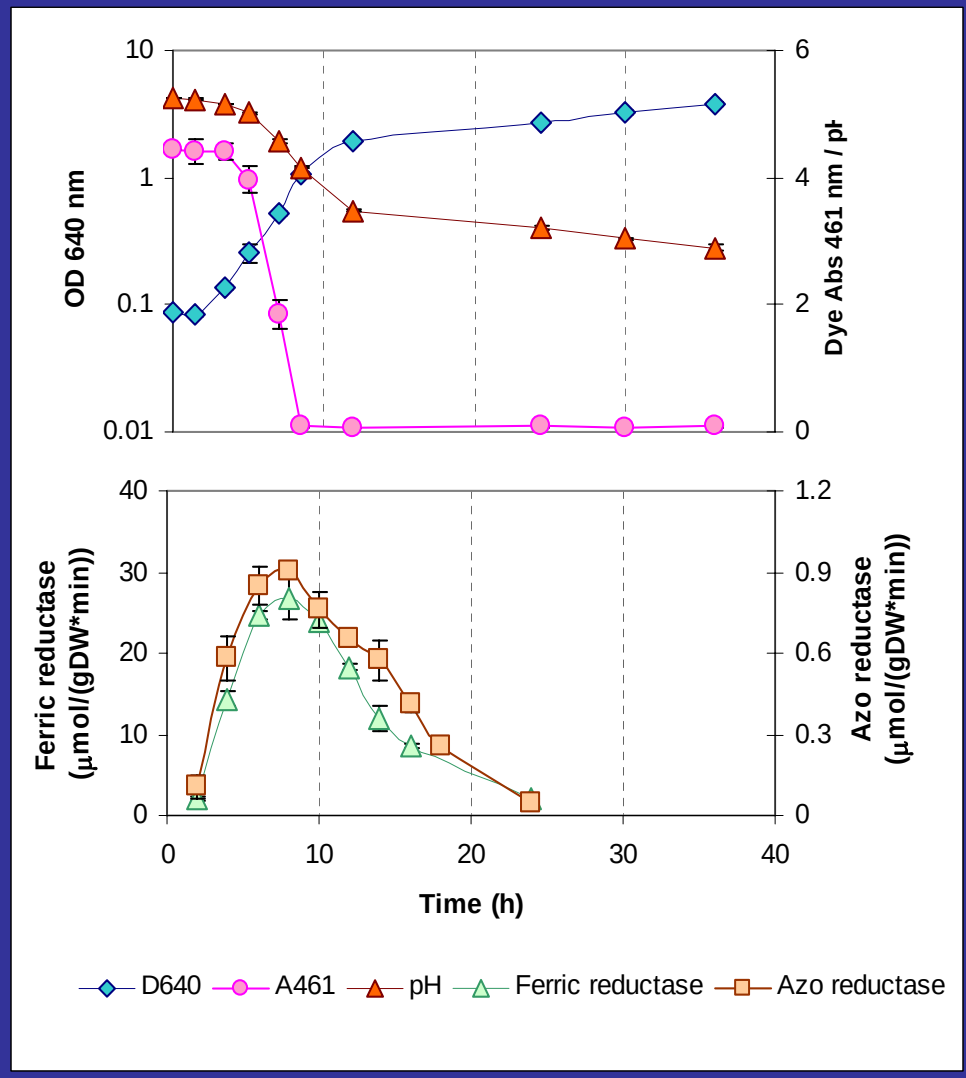


(*In* Shakoury-Elizeh *et al.*, Mol. Biol. Cell, Mar 2004; 15: 1233 – 1243)

Strategies to prove the new functionality of ferric reductase

I. Measurement of both ferric and azo reductase activities along growth and decolourization:

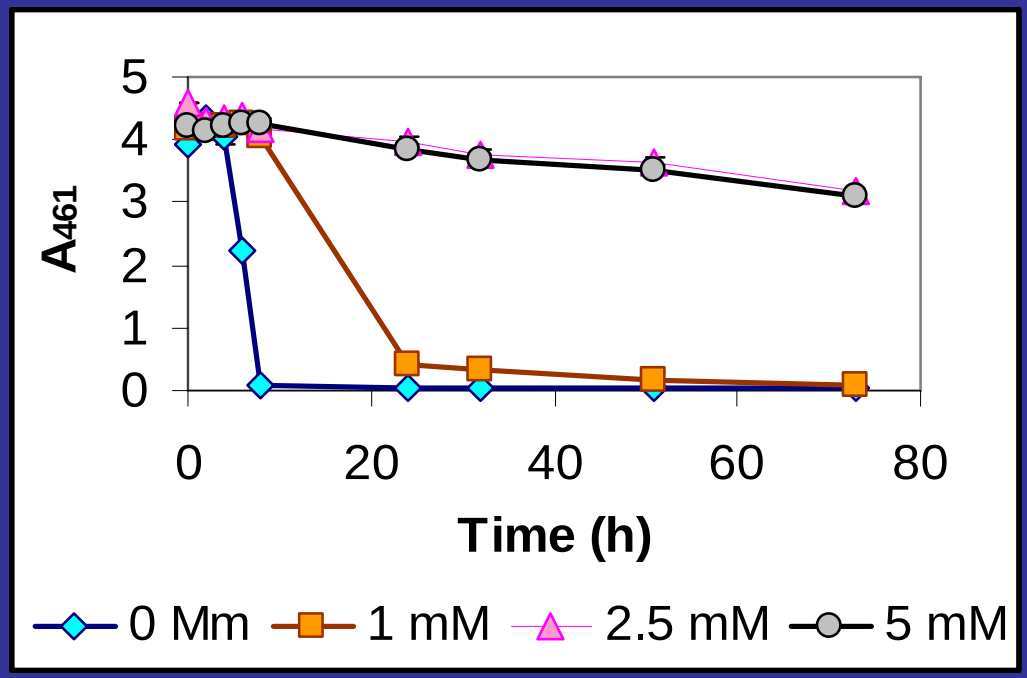
Ferric and azo reductases have **parallel activity curves** with maxima in the late exponential growth phase



Ramalho et al. 2004 **Evidence for two distinct electron donor sites on azo reductase activity in the yeast *Saccharomyces cerevisiae*** Submited to J Biol Chem

Strategies to prove the new functionality of ferric reductase

II. Addition of an known inhibitor of ferric reductase (IRON) to the decolourisation medium:



The addition of iron to the medium inhibits ferric reductase at both transcriptional and post-transcriptional levels (*Lesuisse et al. 1996*) **and delays decolorization**

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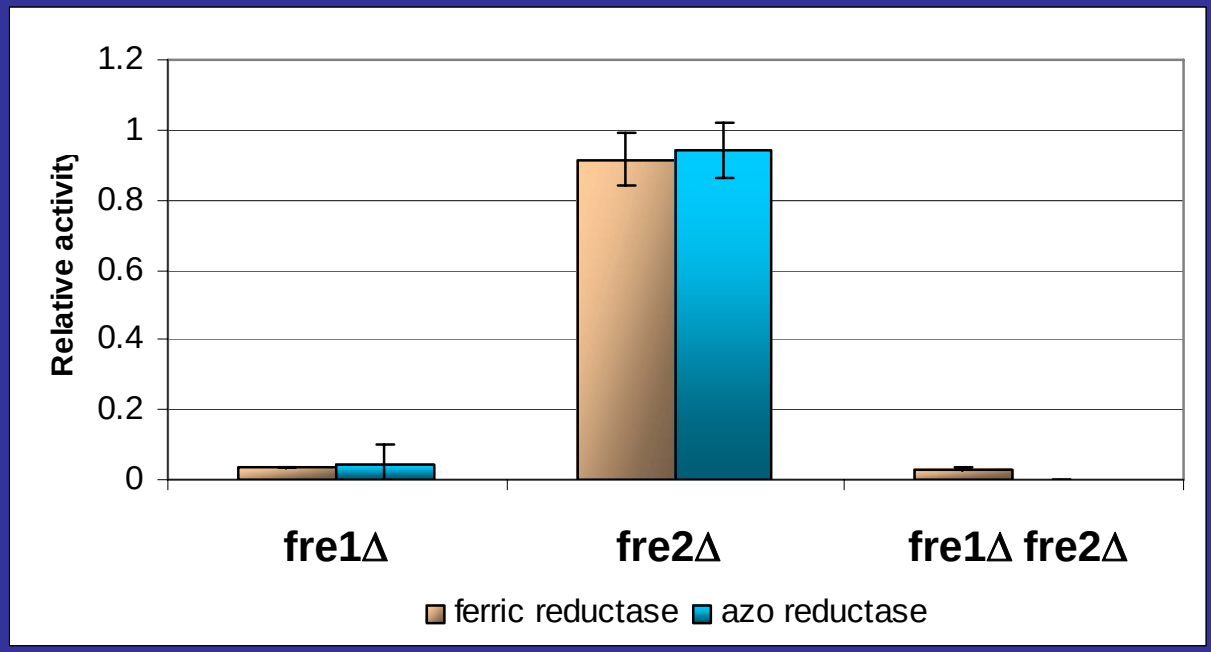
Strategies to prove the new functionality of ferric reductase

III. Deletion of the genes *FRE1* and *FRE2* in the strain: effect on azo and ferric reductase activities



The deletion of *FRE1* gene removes the decolorizing activity; *FRE2* removal does not affect the decolorizing ability, in our conditions

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Strategies to prove the new functionality of ferric reductase

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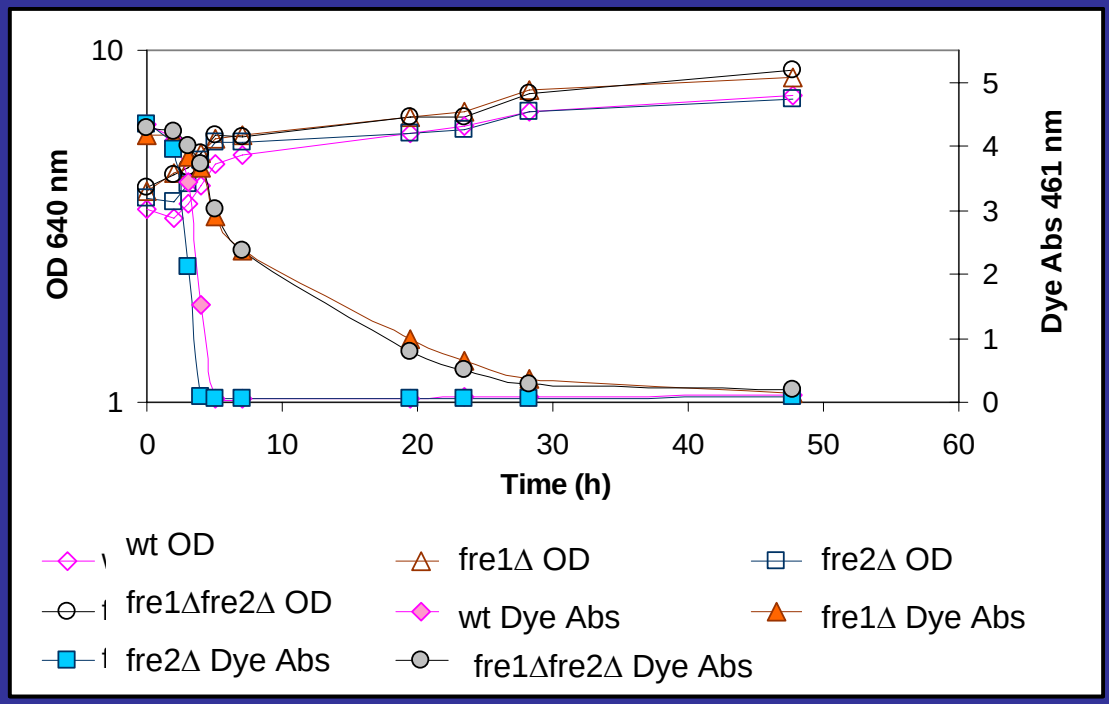

Reduction of decolourising capacity by deletion of *FRE1* and *FRE2*

Fre1p is responsible for the major part of the azo reductase activity of intact yeast cells, but there is an alternative reductase in yeast cells

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Conclusions

- Yeasts are able to reductively decolourize azo dyes in acid effluents
- It is needed an external carbon source, that can be acetate, ethanol or glucose
- Oxygen is needed in limited conditions
- The amines produced are used as carbon and nitrogen sources – the complete mineralization can be achieved
- The enzyme system responsible for the major part of this activity is the plasma membrane ferric reductase

Future perspectives

- Production of a bioreactor to study the effect on the decolourisation process of salts, pH and temperature and test real effluents

- Genetic manipulation of the *Saccharomyces cerevisiae* strain to overexpress the enzyme system

Acknowledgments

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- Thanks to all of you for your attention!